

Morphology of *Arabidopsis* Grown under Chronic Centrifugation and on the Clinostat^{1, 2, 3}

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ABSTRACT

Morphological measurements were made on populations of *Arabidopsis thaliana* grown from seed for 21 days under essentially constant environmental conditions except for the influence of gravitational or centrifugal accelerations. Growth conditions were what had been proposed for experiments in an artificial satellite. Observations are reported for plants grown at normal 1-g upright or on horizontal clinostats and for plants grown on a centrifuge. Increased *g*-force, up to 15 times normal, was found to have significant but small effects on some morphological end points. The plants' sensitivity to the magnitude of the *g*-force was much less than to its vector direction.

Data from centrifuge experiments (which determined the *g*-functions for particular characters) were extrapolated to zero-*g* to predict a set of morphological characteristics of a plant developing in the satellite environment. As an alternative means of predicting properties of a zero-*g* plant, characteristics of plants grown on horizontal clinostats were measured. The results of these two predictive methods were not in agreement.

Clinostat grown plants were morphologically distinct from upright stationary controls. When plants were grown while rotating in the upright position on vertical clinostats they were similar to stationary plants also grown upright, but there were small differences some of which were statistically significant.

maintaining the plant in an inverted position. It can be arranged for the *g*-vector to sweep slowly around the plant axis using a horizontal clinostat, a condition sometimes referred to as "gravity compensation" because over a relatively short time the average *g*-stimulation of the clinostated plant is essentially omnilateral (9).

Of these several test conditions the one that has become available most recently for plant physiological experimentation is that of free fall or weightlessness. The condition, sometimes referred to as "zero-*g*," is interesting not only because it has been exploited least of all in studies of gravitational physiology—and therefore has the attraction of any new, unexplored area for experimentation—but, more fundamentally, weightlessness is patently the appropriate control condition for a variety of tests in which the biological consequences of *g*-input manipulations may be investigated. If one explores an extended range of the *g*-parameter, the most logical control condition is that of zero input of the factor in question—namely, weightlessness. We have under development an experiment to be carried out on board an artificial satellite in earth orbit which will reveal effects of depriving a population of developing plants of all significant acceleration inputs from the environment.

Two important considerations restrict the design of space experiments in which organisms are studied in the absence of any accelerations which might affect those properties of the organisms which are to be measured. First, if the experiment requires that no significant accelerations be imposed unintentionally (by attitude control of the satellite or by other factors) this condition cannot be attained, for technical reasons, in any manned space laboratory already flown or now being designed.⁴ Although most biological experiments proposed for space flight do not require essentially zero-*g* conditions but need only low *g* (about 0.1 or 0.05 *g*), for any which have a much lower requirement (10^{-3} or 10^{-4} *g*) an unmanned satellite must be employed. Therefore, all manipulations involved in the experiment must be automated or carried out by remote control.

The second restriction, although less fundamental, is nevertheless equally determining. In spite of many impressive engineering accomplishments which provided scientific observations and measurements in the space environment, current space age technology and methods of achieving design objectives of a space experiment are not yet equal to the challenge of developing, in a reasonable time and at a reasonable cost, flight hardware and operational procedures which match the level of sophistication of much current biological research. We thought it

An early student of the gravitational responses of plants, Charles Darwin, once complained that "it is impossible to modify in any direct manner the attraction of gravity" and he regretted this restriction on the designs of his physiological experiments (7). Nevertheless, it is possible to modify in several ways the gravitational inputs that a test plant receives from its environment. The direction of the gravitational vector can be altered easily with respect to the organism by displacing the plant from its normal upright position. The *g*-force can be increased by locating the plant on a centrifuge. It can be decreased to nearly zero with the plant in a state of free fall as on an artificial satellite in earth orbit. It can be applied in the opposite direction by

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³ This contribution has the identification, MORFOG-2, as a NASA contractor's technical report.

⁴ This limitation applies especially to NASA manned spacecraft. Although available information on this point is not as complete with regard to Cosmos and Salyut vehicles, there are good reasons to predict that also in those USSR spacecraft intermittent accelerations are substantially in excess of the minimum levels which could prove biologically significant.

prudent to initiate an examination of the morphological consequences of development by plants deprived for the first time in their evolutionary history of directional gravitational information by planning a set of relatively simple observations which would make only minimal demands on the performance of the flight vehicle particularly at the interface between our experimental hardware and the spacecraft. This accounts for our emphasis on in flight photography and postflight examination of the morphology of recovered test plants as a means for identifying ontogenetic dependencies on gravitational acceleration. By our projected space experiment, we propose to examine the growth kinetics and, in particular, certain morphological end points of development in cytologically fixed plants which had completed their life cycle in the weightless condition.

For comparison with those observations, for which there exist no certain predictions, we have explored the effects of a variety of *g*-conditions as mentioned above. We present the results of those earth based "control" studies at this time (rather than when data become available from the space flight experiment) chiefly because our ground-based morphological studies may have an intrinsic interest as they not only reveal the extent of morphological flexibility that our test plants exhibited under several different kinds of *g*-input conditions but, they also provided some bases for predicting morphological end points of development of a population of plants grown under weightlessness.

MATERIAL AND METHODS

Our experimental organism was *Arabidopsis thaliana* (L.) Heynh., the same species as is planned for satellite flight experiments to observe plant development under conditions of reduced *g*-force (including weightlessness). Apart from its well known advantages for genetic work, this species has been found advantageous for studies in gravitational physiology (9). Its role as a promising object for experiments in aerospace biology has been reviewed by Ivanova (13).

Test plants were cultured aseptically at 24 ± 1 C on agar containing mineral nutrients with the addition of sucrose (2 weight %) and glutamic acid (0.002 weight %). Seeds were wet with 95% ethanol for no longer than 1 min and surface-sterilized for 3 min in 1% H_2O_2 . The seeds were planted in individual Pyrex glass modules (Corning No. 6900 "cloud and pour test jar") which were about 3.6 cm in diameter and 13 cm tall and contained 20 ml of sterilized nutrient agar. The initial pH was 6.1. After planting, modules were sealed with a small sheet of 0.5 mil Saran wrap pressed tightly over the module rim and retained by rubber bands. The water loss from a module thus sealed with Saran wrap was at the negligible rate of about 1 mg/day.

The modules were illuminated continuously by light banks made up of Sylvania GroLux Wide Spectrum fluorescent lamps. The nominal light intensity was 162.5 ft-c, a specification that was established empirically. The light intensity was measured either by a General Electric photographic exposure meter (Model 213) or by a Clairex photoconductive cell (CL 605) referred by appropriate calibration to the GE 213 meter which was itself calibrated at the GE Standards Laboratory. Since the light source had a unique emission spectrum (2) the foot candle readings of the GE 213 meter were only relative. The absolute intensity of light incident on the seedlings was 6.6×10^3 erg cm^{-2} sec^{-1} as measured with a YSI-Kettering radiometer, Model 65.

Accelerations were produced by a centrifuge designed for continuous operation. This is a symmetrical or double armed rotating machine with two pairs of swinging cradles. The use of swinging cradles ensured that the force vector (resultant of centrifugal and gravitational force) was perpendicular to the floor of the cradle and to the long axis of the plants. The

maximum working diameter to the limits of the outer cradle floors is 693 cm. The design limit for rotation rate (in terms of resultant *g*-force attainable in the outer cradles) corresponded to 20 *g*. Long term stability of the *g*-force, when the centrifuge was operating unattended, produced a "worst case" error of $\pm 3\%$ at 20 *g*, $\pm 4\%$ at 10 *g*, and $\pm 5\%$ at 5 *g*. The *g*-levels applied to the experimental payloads were determined mainly by the rotation rate. Placement of the test organisms within the space of the payload cradles was held constant. Since a pair of cradles was available at each of two radii, for a given centrifuge speed, two *g*-levels could be applied to different payloads in a single test.

Planted modules were inserted into individual clinostats ganged together so that 24 operated from the same drive motor. The banks of clinostats, each covering about 160 cm^2 , were housed in boxes fitted with lamps, a ventilating fan, and monitors for light intensity and for temperature. All rotating clinostats turned at the rate of either 2 or 2.18 rpm. When plants were grown at 1 *g*, the boxes were not located on the centrifuge. For most experiments reported here, the upright plants, whether they were centrifuged or not, were not rotated.

Usually within a few hours after seeds were planted, centrifugation was begun and continued without interruption for 21 days. In some tests, it was convenient to refrigerate the planted modules over night at 2 C before starting the centrifugation. In preliminary tests, we determined that this cold treatment had no significant effect on any of the developmental end points we measured. In fact, germination could be delayed by incubation at 2 C for over a week without a detectable vernalization effect on subsequent development.

At the conclusion of a test, plants were chemically preserved by flooding each module with a cytological fixative Karpechenko's fluid (14). Subsequent microscopic examination and dissection were performed in order to obtain a set of measurements of morphological end points of development under the different conditions of gravitational information supplied to the test specimens.

Where appropriate, statistical analyses of the data were performed by conventional methods. In cases where data populations were small, the significance of differences between means were determined by Student's *t* test. For the errors associated with the extrapolation of linear data trends, we depended on the method given by Ezekiel (8).

Several years ago at the NASA Ames Research Center we carried out a series of three pilot experiments using the 17.6-m diameter Biosatellite centrifuge. Test conditions were similar to those employed in the work reported here. The main differences were in the range of clinostat rotation rates, a lower light intensity, and seed from a different harvest. The results of those pilot studies were consistent with those reported here from similar but more extensive experiments in our own laboratory. It did not seem advisable to pool the results obtained earlier at the Ames laboratory with our more recent results. Therefore, all findings reported here represent work carried out in our Philadelphia laboratories. The Ames results were the basis of two reports (5, 6), parts of which have been confirmed and extended by the present contribution.

RESULTS AND DISCUSSION

A. Plants Developed in Upright Stationary Position at Normal Gravity (1 *g*). The customary reference condition for botanical experiments in which the gravitational factor was manipulated has been that of a plant growing in its normal upright position in the earth's gravitational field (10 m sec^{-2} , conveniently designated as 1 *g*). Populations of *Arabidopsis* plants cultured under our standardized laboratory conditions germinated (radicle evident) between 25 and 44 hr after planting. On average the first leaf appeared in just under a week (163 hr), and

a set of about 6 rosette leaves was formed by the end of the 2nd week. Rarely as many as 8 rosette leaves developed, but on average only 5.8. were formed. Bolting began about day 14, attained a maximal rate on day 19 of 4 mm hr⁻¹, and continued at a decreasing rate to day 30 and beyond. After the start of rapid elongation of the flowering stem, the appendages (usually beyond the sixth) were bracts rather than rosette leaves. Early bolting usually resulted in fewer leaves and more bracts; delayed bolting usually was associated with more rosette leaves and fewer bracts. The average age to the first appearance of a white flower was just over 26 days. Figure 1 illustrates the time course of these features of *Arabidopsis* development during 29 day periods of observation (average of two experiments; total of 21 individual plants).

As originally planned, the first NASA space mission on which we could include an *Arabidopsis* experiment was limited to 21 days—a period which we considered minimal rather than optimal for observations on the ontogeny of this species. Therefore, we initiated a series of ground-based studies of various morphological end points of *Arabidopsis* development after 21 days of growth.

In nearly all our tests with *Arabidopsis*, we included plants that were maintained in their normal upright positions and we accumulated thereby a large number of measurements of developmental end points of 21-day-old control plants. Table I shows the averages of those measurements for seven gross morphological features.

Some characters were intrinsically more variable than others, as shown by the different coefficients of variability; plant height

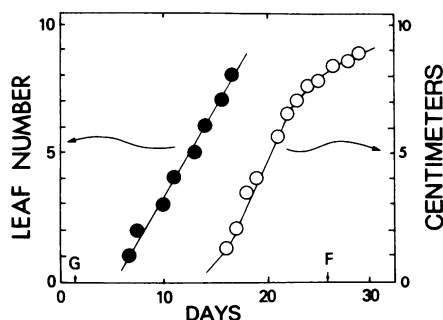


FIG. 1. Time course of development of *Arabidopsis* plants. Seeds were planted at day zero. Average plant height (○); ages at which successive rosette leaves appeared (●); G: time of germination. F: time of flowering.

Table I. Mean Values for Morphological End Points of Development of *Arabidopsis* Plants Grown Upright Stationary at Normal Gravity. Period of growth was 21 days; number of plants was 106.

Character	No. of Measurements	Mean Value	Coefficient of Variability ¹	Calculated SE for Mean of 10 Plants ²
		mm	%	
Leaf length	528	9.76 (±0.13) ³	27.6	3.6
Petiole length	528	4.97 (±0.07)	34.7	4.5
Blade length	528	4.79 (±0.06)	31.3	4.1
Blade width	528	2.77 (±0.03)	21.5	2.8
No. of leaves	106	5.83 (±0.07)	11.6	3.7
Hypocotyl length	106	5.27 (±0.12)	22.7	7.2
Flower stem length	106	50.1 (±2.1)	43.8	13.9

¹ SD/mean × 100.

² SE expressed as per cent of mean.

³ Numbers in parentheses are SE which were calculated by the formula

$$\sqrt{\frac{\sum(x - \bar{x})^2}{n(n-1)}}$$

(flower stem length) was the most variable and number of rosette leaves the least. From these data it was possible to predict the precision with which any of these characters might be determined on a much smaller population. In the projected satellite experiment mentioned above, the test population will include 10 plants. The conversion shown in equation 1 was used to calculate from the coefficient of variability (v) an expected standard error (SE) of the mean measurement (m) on a population of 10 plants (58.3 leaves):

$$SE = \frac{m}{\sqrt{vn}} \quad (1)$$

For convenience, the standard error is expressed as a per cent of the mean. This estimate for each of the seven characters measured is given in the last column of Table I. If the same biological variability should occur under weightlessness, we may expect to find that statistical variations (represented by the standard errors of means) in the quantities measured on only 10 plants grown on board a satellite should amount to less than 5% of the mean measurement for all but two characters, hypocotyl length and flower stem length, for which standard errors of about 7 and 14% of the respective means might be expected.

B. Plants Developed at Increased g -Levels Achieved by Chronic Centrifugation. *Arabidopsis* populations were grown on the centrifuge at each of a series of 11 g -levels covering the range 1 to 15 g . Over the 15-fold range of chronically imposed g -forces, the plants exhibited remarkably small differences at age 21 days. Figure 2 shows a sample of these data (open circles), which established g -functions for the several characters of interest. In Figure 2A, it is apparent that the regression line, fitted by the method of least squares, has a small negative slope. In Figure 2B, the regression line has nearly zero g -dependence.

The data for these and other characters are presented numerically in Table II. It is evident from the relatively low values of the linear regression coefficients for any of the characters measured (column II) that the intensity of the g -force in the range 1 to 15 g was not an important determinant of development. For all characters an increase in g -level reduced the measured values but in no case was the decrease in excess of -2% per g -unit increment.

The regression coefficients of Table II were so close to zero that we felt it was worthwhile asking the statistical question, were the values of the characters significantly correlated with the imposed g -level? Column III, Table II shows the several correlation coefficients, and column IV shows which coefficients were

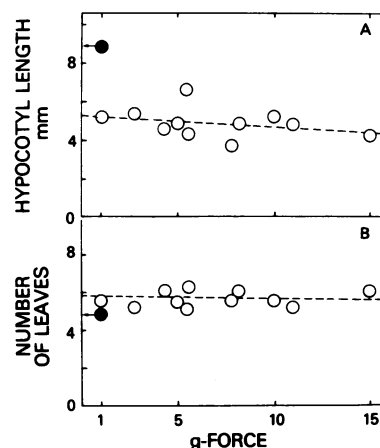


FIG. 2. Effects of increased g -force on two morphological characters of *Arabidopsis* plants grown for 21 days under continuous centrifugation. A: Relation between hypocotyl length and chronic g -force (○); B: relation between number of rosette leaves and chronic g -force. A and B: respective measurements on clinostated plants (●). (See section E under "Results" for discussion.)

significantly different from zero. Five of the characters showed correlations with g -level which were significant at the 1% level; one at the 5% level. Only one character (number of leaves) was not significantly correlated with the magnitude of the imposed g -force.

As organisms which are patently sensitive to an acceleration as low as $10^{-3} g$ (11, 17), higher plants might be expected to respond in more than marginal degree to abnormally high accelerations—in this case up to 1500% above normal. However, from other kinds of experiments on a different species, we have reason to believe that plants may be highly sensitive to the direction of the g -vector but, above some threshold not yet determined, insensitive to its magnitude (3). The data of Table II are consistent with that view.

C. Extrapolation of g -Functions of Plant's Morphological Characteristics to Predict Values to be Expected at Zero- g . One reason for making measurements on plants grown at different g -levels was to establish g -functions of the several morphological end points of development. By extrapolation of those functions to the ordinate (zero- g) it is possible to arrive at a set of predictions which might apply to a plant grown in the weightless environment of an artificial satellite. Table III gives the results of those extrapolations—i.e. the respective values calculated for zero- g . These values constitute meaningful predictions of the characteristics of a plant grown under weightlessness only if the linearity of the g -functions is taken for granted.

D. Plants Developed on Horizontal Clinostats. *Arabidopsis* plants grown from seed for 21 days on horizontal clinostats were at approximately the same stage of maturity as plants grown in the normal upright position. For the characters we are reporting here, some differences between upright stationary and clinostated plants were pronounced. Plant height was much reduced and hypocotyls greatly elongated by clinostating. Also the number of rosette leaves was significantly reduced on the clinostat.

Table II. Effect of g -Level on Mean Values for Morphological End Points of *Arabidopsis* Plants Grown under Continuous Centrifugation

The resultant g -force was at all times parallel with the longitudinal axis of the test plants. Period of growth at specified g -levels was always 21 days. Number of plants was 296.

Character	I. No. of Measurements (n)	II. Regression Coefficient (b) ¹	III. Correlation Coefficient (r)	IV. Probability That r Differs from Zero only by Chance (p)
Leaf length	1464	$-0.172(\pm 0.018)^2$	-0.239	<0.0001
Petiole length	1464	$-0.094(\pm 0.011)$	-0.220	<0.0001
Blade length	1464	$-0.077(\pm 0.009)$	-0.213	<0.0001
Blade Width	1464	$-0.026(\pm 0.004)$	-0.173	<0.0001
No. of leaves	296	$-0.014(\pm 0.014)$	-0.062	0.2923
Hypocotyl length	296	$-0.058(\pm 0.020)$	-0.166	0.0045
Flower stem Length	296	$-0.753(\pm 0.307)$	-0.141	0.0156

¹ Linear regression line fitted by method of least squares.

² Numbers in parentheses are SE.

Table III. Predicted Values of Morphological Characters of *Arabidopsis* Plants to be Grown under Weightlessness and Determined by Linear Extrapolations

Character	Value by Extrapolation at Zero- g
Leaf length (mm)	$10.937 (\pm 0.007)^1$
Petiole length (mm)	$5.327 (\pm 0.003)$
Blade length (mm)	$5.058 (\pm 0.002)$
Blade width (mm)	$2.887 (\pm 0.0003)$
No. of leaves	$5.807 (\pm 0.005)$
Hypocotyl length (mm)	$5.239 (\pm 0.006)$
Flower stem length (mm)	$50.421 (\pm 2.054)$

¹ Numbers in parentheses are SE.

Leaf shape characters were altered only slightly or not at all. Table IV gives average measurements for plants grown on clinostats. The last column of the table shows how these averages compared with the corresponding mean measurements of plants grown in the upright position (without rotation).

E. Does Extrapolation from Data Obtained at Higher g -Levels Predict Same Values for Plant Characters at Zero- g as Data from Clinostat-grown Plants? In section C we presented the rationale for predicting characteristics of a plant grown under zero- g conditions by extrapolation from data collected over a range of g -levels above unity. We have no way of making sure, in advance of an experimental test of the procedure, that the g -function of any given character will follow the same trend below 1- g as it does at higher g -levels. However, that kind of uncertainty plagues most extrapolation procedures whenever the function is only empirical and has no particularly convincing theoretical support. In that light we presented in Table III a set of predictions, obtained by extrapolation, for a plant grown under weightlessness.

An alternative and perhaps equally shaky set of predictions of characteristics of a zero- g plant is, according to some authorities, the array of character measurements obtained with plants grown on horizontal clinostats (1, 15). Of course, the time-averaged omnilateral gravity stimulation of the clinostated plant is fundamentally different from no stimulation at all, but it may be that the effects on plant development of omnilateral and therefore perhaps "compensating" stimulations would closely simulate the developmental influence of weightlessness.

We need not labor the point that of the two methods for predicting the characteristic of a zero- g plant—extrapolation or clinostating—neither is theoretically very secure; our purpose here is to apply both methods and compare the two sets of predictions. In Table V the two kinds of predictions are shown side by side. The last column of the table gives, for each character compared, the probability that the difference could have been accounted for by chance alone. In the case of mean petiole length the difference was significant at the 5% level; for each of the remaining characters the difference was significant at the 1% level. Two of the comparisons made in Table V are illustrated in Figure 2 where the values for plants grown on horizontal clinostats at 1- g are symbolized by solid circles with attached arrows. In one case (B) the value for clinostated plants lies not far from the value at which the dashed regression line from centrifuged plants intersects the ordinate (at zero- g); in the other example (A) the value for clinostated plants lies well above the ordinate intersection of the data from centrifugation experiments.

For more convenient graphic comparisons, the two sets of

Table IV. Mean Values for Morphological End Points of Development of 21-day-old *Arabidopsis* Plants Grown on Horizontal Clinostats

Clinostats were rotating at 0.5, 2.18, or 2.5 rpm in different experiments.

Character	No. of Measurements	Mean Measurements for Clinostated Plants	Mean Measurements as Per Cent of Value for Upright Stationary Plants ¹
			%
Leaf length (mm)	850	$9.86 (\pm 0.24)^2$	101
Petiole length (mm)	850	$5.05 (\pm 0.13)$	102
Blade length (mm)	850	$4.67 (\pm 0.12)$	97
Blade width (mm)	850	$2.76 (\pm 0.05)$	100
No. of leaves	176	$4.88 (\pm 0.09)$	84
Hypocotyl length (mm)	176	$8.91 (\pm 0.46)$	169
Flower stem length (mm)	176	$37.76 (\pm 2.17)$	75

¹ Data for upright plants from Table I.

² Numbers in parentheses are SE.

zero-*g* predictions were normalized by the respective mean value of each character for plants grown in the normal stationary, upright position (as recorded in Table I), *i.e.* ratios were calculated for each character for each predictive method,

$$\frac{E_0}{m_1} \text{ and } \frac{C_0}{m_1}$$

where E_0 refers to a character value predicted at zero-*g* by the extrapolation method, C_0 to a character value predicted at zero-*g* by the clinostat method, and m_1 to a value measured on upright stationary plants grown at 1-*g*. These ratios, plotted as per cent departures from respective measurements at 1-*g*, are shown in Figure 3.

F. Does Rotation on a Vertical Clinostat Influence Plant Development? Most clinostats have been designed to rotate the test plant about its longitudinal axis although other axes also have been used. The plant usually is rotated on the clinostat in a horizontal orientation even though angles with respect to the plumb line other than 90° have been employed for special pur-

poses. We are concerned here only with angles of inclination which were 90° and 0°. The clinostat imposes several special conditions which might influence plant growth. Displacement of the plant away from the plumb line, rotatory motion around the axis, centrifugal acceleration resulting from the rotation, and vibrations introduced from the clinostat drive mechanism are separate factors each of which might exert an influence of its own.

Perhaps the influence of rotation alone or "vertical rotation" (with the plant axis in coincidence with the plumb line) is the factor most important to examine because many experimenters have used vertical stationary control plants to compare with plants rotated horizontally on clinostats on the assumption that rotatory motion *per se* could have no effect. In a few cases investigators have used two sets of vertical controls, those which were rotated about the plants' vertical axes and those which were stationary. In some cases the two kinds of controls showed no significant difference for the character studied (12), but in other cases reported from the same laboratory the authors noted that certain properties of vertical stationary and vertical rotated plants were not the same. Statistically significant differences, interpreted as "effects of rotation *per se*," included increased phototropic responsiveness (16), incidence of multiple nucleoli in cells of carrot root callus cultures (10), and enhanced geotropic responsiveness in *Avena* (9). In our own work with *Arabidopsis*, we observed during preliminary experiments that vertical stationary and vertical rotated control plants differed significantly in some characters we measured (4). Although some of these diverse effects of rotatory motion appeared small, others were not. For example, in the case of phototropic responsiveness of *Avena* (16), the effect of rotatory motion in the vertical position was about as large (and of opposite sign) as the overall effect of rotation on the clinostat. Also in the case of a 4-fold increase in incidence of cells with multiple nucleoli (10), one-third was accounted for as an effect of rotation alone. By such results we are warned that vertical stationary plants may or may not develop differently, both physiologically and morphologically, from those rotated about the vertical axis. This further suggests that careful thought be given to exactly what kind of "controls" would be most appropriate for tests using horizontal clinostats.

We made several kinds of systematic comparisons of *Arabidopsis* plants grown under three conditions: on horizontal clinostats, vertical but stationary, and vertical rotating. Table VI shows the results of one such experiment from which effects of clinostating were evident. It also shows that rotation of vertically oriented plants caused them to develop in some respects differently from the stationary vertical controls. These data make it possible in principle to separate a motion effect, *M* (attributable to rotation alone), from a position effect, *P* (due to displacement of the plant axis from the plumb line). The sum of motion and position effects constitute the overall clinostat effect, *C*. Thus,

$$M + P = C \quad (2)$$

Table VI. Morphological End Points at Day 21 of *Arabidopsis* Development under Different Conditions of Plant Rotation

Rotation rate was 2.18 rpm.

Character Measured	Rotation on Horizontal Axis Clinostat		Rotation on Vertical Axis Clinostat		Vertical Position without Rotation	
	<i>n</i>	mean \pm SE	<i>n</i>	mean \pm SE	<i>n</i>	mean \pm SE
Leaf length (mm)	97	7.82 \pm 0.42	107	8.21 \pm 0.18	99	8.57 \pm 0.24
Petiole length (mm)	97	4.27 \pm 0.27	107	3.64 \pm 0.11	99	4.26 \pm 0.13
Blade length (mm)	97	3.55 \pm 0.17	107	4.56 \pm 0.10	99	4.31 \pm 0.13
Blade width (mm)	97	2.14 \pm 0.08	107	2.53 \pm 0.04	99	2.35 \pm 0.04
No. of leaves	21	4.81 \pm 0.20	23	5.13 \pm 0.21	20	5.40 \pm 0.17
Hypocotyl length (mm)	21	9.01 \pm 0.81	23	6.09 \pm 0.38	20	6.38 \pm 0.30
Flower stem length (mm)	21	12.63 \pm 2.20	23	57.11 \pm 3.00	20	47.72 \pm 4.09

Table V. Comparison of Predictions of Characteristics of Plant Grown in Weightlessness

Character To Be Predicted	Most Probable Value Obtained by Extrapolation from Higher <i>g</i> -Levels ¹	Most Probable Value Obtained by Simulation with Clinostat ²	Probability That Differences Were due to Chance
Leaf length (mm)	10.94	9.86	<0.0001
Petiole length (mm)	5.33	5.05	0.01 < <i>P</i> < 0.05
Blade length (mm)	5.06	4.67	<0.002
Blade width (mm)	2.89	2.76	<0.01
No. of leaves	5.81	4.88	<0.0001
Hypocotyl length (mm)	5.24	8.91	<0.0001
Flower stem length (mm)	50.42	37.76	<0.0001

¹ Values from Table III.

² Values from Table IV.

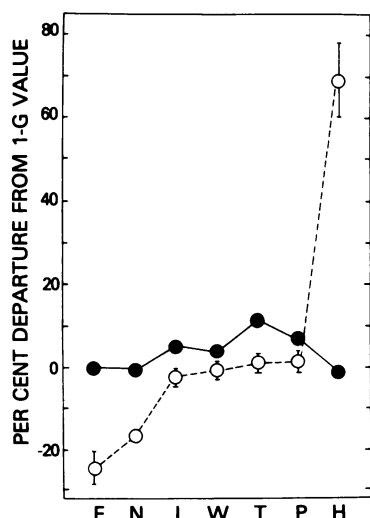


FIG. 3. Comparison of the results of two methods for predicting quantitative characters of *Arabidopsis* plants to be grown under satellite weightlessness. Measured characters are aligned in arbitrary sequence. F: flower stem length; N: number of leaves in rosette; L: leaf blade length; W: leaf blade width; T: total leaf length; P: petiole length; H: hypocotyl length. All measurements were normalized to respective values of plants grown upright stationary at 1-*g*. Clinostated plants (●); extrapolations from centrifuged plants (○). Ordinate, per cent departure from value for plants grown upright stationary at 1-*g*. Error bars indicate ± 1 SE unit. For clinostated plants error bars lie within area of symbols.

where each term, if different from zero may be either a positive or a negative quantity. We define these terms as follows:

$$M = \frac{r - s}{s} \times 100$$

$$P = \frac{c - r}{s} \times 100$$

$$C = \frac{c - s}{s} \times 100$$

r = measurement of a given character of plants exposed to rotation in upright orientation (vertical clinostat); s = measurement of the given character of plants grown vertically without rotation; c = measurement of the given character of plants grown on horizontal clinostats. From these definitions it follows that if a vertical rotation effect is detected (i.e. if $M \neq 0$), then C must consist of two components, M as well as P . Of course, M and P could be of different sign and C could turn out to be smaller than either.

Following this reasoning, the data of Table VI were used to calculate M , P , and C for *Arabidopsis* plants. The results are shown in Figure 4. Wherever the motion effect was negligible the overall clinostat effect was, of course, nearly the same as the position effect. However, where the motion effect was substan-

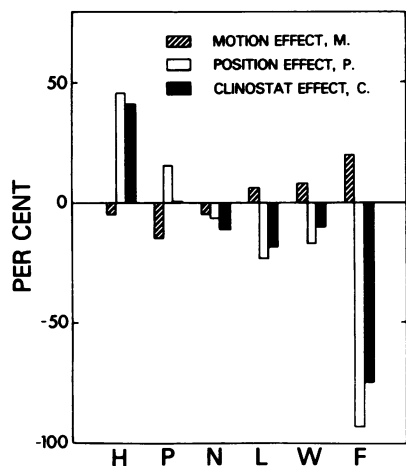


FIG. 4. Calculated motion effects, position effects, and overall clinostat effects on *Arabidopsis* development. Characters aligned in arbitrary sequences are identified by letters as indicated in Fig. 3 legend. For each character, M is plotted as the first bar, P as the second bar, and C as the third bar of the group. For method of calculating M , P , and C , see section F under "Results".

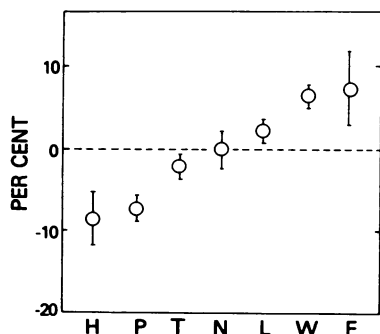


FIG. 5. Effects of rotation in the vertical position on morphological characters of *Arabidopsis* development. Characters aligned in arbitrary sequence are identified by letters as indicated in Fig. 3 legend. Ordinate, value of M , rotatory motion effect, calculated as described in text. Error bars represent the limits of ± 1 SE unit.

tial, it was able to contribute to or counteract the position effect as it influenced the development of clinostated plants. We note that for *Arabidopsis* very large motion effects were not encountered. Statistical analysis of the data in Table VI revealed that only for petiole length and blade width were motion effects significant at the 1% probability level.

We carried out two additional tests, specifically designed to maximize our chance of observing a motion effect, in which all test plants were vertical but half of them were rotated. The experiments employed banks of clinostats in which alternate units were held stationary; thus, rotating and nonrotating plants were side by side in as nearly the same environment as could be achieved. The results confirmed that there were significant but no very large effects of rotation in the vertical position. The largest effect detected in the combined results of those two tests was a 9% enhancement of the leaf blade width, an effect that was statistically significant at the 1% probability level.

When the data from all relevant tests were pooled, we obtained the array of vertical rotation effects shown in Figure 5. The calculated effects of vertical rotation are plotted in the arbitrary order of increasing values of M . A statistical analysis of the data on which Figure 5 was based showed that only two of the characters had M values significantly different from zero. Petiole length and leaf blade width both exhibited rotation effects which were significant at the 1% probability level.

It is important to note that for all *Arabidopsis* characters we measured the effect of motion (rotation in vertical position) was small whether statistically significant or not. The largest negative effects we found were about 8% inhibition of petiole length, 8% reduction of leaf length/width ratio, and 8% shortening of the hypocotyl. The largest enhancement effects were on leaf blade width (6%) and on flowering stem length (7%). Since clinostat effects mostly ranged from about +35% down to about -25% for different characters, it is evident that the effects of horizontal position (here termed P) is the chief component of the overall clinostat effect (C in present notation). However, motion effects (M) that can be calculated from data reported for other plant systems were sometimes larger than we found for *Arabidopsis*. From data on phototropic responsiveness of *Avena* seedlings (16), $M = +5.1\%$, $P = -10.3\%$, and $C = -5.2\%$. In the case of the incidence of nucleoli in cells of carrot root tissue culture, C was increased nearly 4-fold by rotation of the culture on the horizontal clinostat and about two-thirds of the increase was due to P , one-third to M (10). In a study of amyloplast size and number, M was found to be zero, since P was equal to C (12). Even though some motion (vertical rotation) effects evidently are far from negligible, it would be difficult to defend any sweeping generalizations at the present state of exploration of the subject. The existence of some large effects of this kind remains as a warning that the horizontal clinostat is not a simple device which only eliminates the directional effect of gravity from the test plant's environment.

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